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Microwave Assisted Efficient Synthesis of 5H-dibenzo [b, i] xanthene-tetraones by using K_2CO_3 as base catalyst and their Biological Evaluation

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Abstract: A rapid and highly efficient synthesis of 5H-dibenzo [b, i] xanthene-tetraones derivatives by condensation of aromatic aldehydes and 2-hydroxy 1, 4-naphthaquinone using K_2CO_3 as base catalyst and its bio-computational studies is reported. The advantages of this method includes good to excellent yields, operational simplicity, shorter reaction time, easy work up procedures and eco-friendly reaction conditions. The synthesized compounds were screened for anti-bacterial assay using Staphylococcus aureus and Escherichia Coli as bacterial strains and anti-inflammatory screening using Gelatine Zymography.

Keywords: 2-hydroxy 1, 4-naphthaquinone, aromatic aldehyde, K_2CO_3, Microwave Irradiation, Anti-bacterial, Anti-inflammatory etc.

Introduction

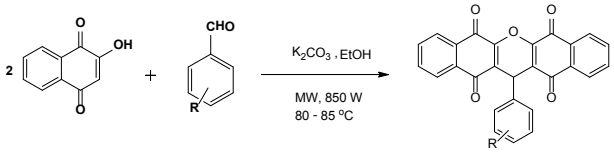
The major objective of organic and medicinal chemist is to design and synthesise chemical entities having high therapeutic and medicinal value. Major Antibiotics are rendered ineffective in curing infectious diseases caused due to bacteria and microbes, due to high resistance of pathogenic bacteria. So it is necessary to design a new class of molecules having resistance against bacteria and fungi. [1-5]

2-Hydroxy-1,4-naphthoquinone, or lawsone, or hennotannic acid, is one of the simplest naturally occurring naphthoquinones, occurring in many natural products and plays key role as a synthetic intermediate for the preparation of heterocyclic compounds with interesting biological properties such as anticancer, antibacterial, antifungal and anti-inflammatory agents[6-8].A bioactive molecule originates from the xanthenes containing heterocyclic quinone group. Also they show useful spectroscopic properties and are used as dyes as well as in laser technologies [9]. Thus, the synthesis of 13-aryl-5*H*-dibenzo [b,i]xanthene-

5,7,12,14(13*H*)-tetraones by using green approach is need of the hour. Xanthene moiety containing quinones as heterocycles shows interesting biological activities. [10, 11] among quinones, naphthoquinones have been found to possess good fungicidal activities [12]. When quinines fused with oxazole or thiazole, it shows good bactericidal activity [13].

The syntheses of 5H-dibenzo [b, i] xanthene-tetraones by addition of two molecules of 2-hydroxy 1,4-naphthaquinone and one molecule of aromatic aldehyde using variety of catalysts such as ferric chloride hexahydrate [14], basic ionic liquid from ethan-1,2-diyl-bis (hydrogen sulfate) and 1,8-diazobicyclo[5.4.0] undec-7-ene [15], phosphomolybdic acid [16], $Fe_3O_4@SiO_2–SO_3H$ [17], acetic acid [18], p-toluene sulfonic acid [19], acidic ionic liquid 1-butyl-3-methylimidazolium hydrogen sulphate [20].

In this work, we report a new eco-friendly synthetic approach for the syntheses of 5H-dibenzo [b, i] xanthene-tetraones using K_2CO_3 as base catalyst under microwave irradiation **(Scheme 1).**



Scheme 1 K_2CO_3 base catalyzed synthesis of 5H-dibenzo [b, i] xanthene-tetraones

Material and methods

All the chemicals were obtained from Sigma Aldrich and Spectrochem and used without further purification. All reactions were performed in the borosil round bottom flask, volume 25 mL. Analytical thin layer chromatography was performed using TLC pre-coated silica gel 60 F_{254} Merck (20 × 20 cm). TLC plates were visualized by exposing to UV light. Microwave reactions were carried out in a microwave synthesizer system (850W power; Cata R System). Melting points were taken in an open capillary and are uncorrected. ^{1}H NMR and ^{13}C spectra were obtained using AV 400 Bruker 400 MHz NMR instrument. Chemical data for protons are reported in parts per million (ppm, scale) downfield from tetramethylsilane (TMS) and are referenced to the residual proton in the NMR solvent (DMSO: δ 2.5). Antibacterial and Anti-inflammatory susceptibility assay carried out at Central Research Laboratory, Maratha Mandal, Belgaum, Karnataka.

General procedure for the synthesis of 13-aryl-5*H* dibenzo[b, i] xanthenes5, 7,12,14(13*H*)-tetraones:

A mixture of 2-hydroxy 1,4-naphthaquinone (2mmol) and aromatic aldehyde (1 mmol) and K_2CO_3(20 mol%) as base catalyst in ethanol(3mL) were irradiated in microwave synthesizer system at 850W (80^{o}C-85^{o}C) for 180 sec. Upon completion, the reaction mixture was cooled to room temperature. Then the precipitated products were filtered and washed with hot water (5mL) and cold ethanol (5mL). Further purification was followed by crystallization from ethanol. **(Scheme 1).**

Spectral data of a representative compounds 13-(4-Chlorophenyl)-5H-dibenzo [b,i] xanthene- 5,7,12,14(13H)-tetraone [Table 3, entry 1, Code No. – KNP 103]

^{1}H NMR (400 MHz, DMSO-d6):δ 6.67 (s, 1H), 7.12–7.98 (m, 12H). ^{13}C NMR (100 MHz, DMSO-d6): (ppm) 32.55, 122.01, 125.07, 125.67, 127.55, 128.69, 130.92, 131.84, 133.20, 133.71, 140.54, 158.85, 182.26, 183.48. FT-IR ν_{max} cm^{-1} : 2908, 1666, 1595, 1278, 1089,727. Orange powder (90%); mp: 330–332 ^{o}C

13-(4-Methylphenyl)-5*H*-dibenzo[b,i]

xanthene-5,7,12,14-(13*H*)-tetraone [Table-3, entry 2, Code No.- KNP 105].

^{1}H NMR (400 MHz, DMSO-d6):δ 2.21 (s,3H), 6.65 (s, 1H), 6.88–7.98 (m, 12H).^{13}C NMR (400 MHz, DMSO-d6): (ppm) 20.45, 32.59, 125.00, 125.65, 126.67, 128.25, 130.90, 131.76, 133.28, 133.38, 133.67,183.61. FT-IR ν_{max} cm^{-1} :3090, 1670, 1595, 1176,727.
Red powder (82%), m.p. 305 - 307 ºC

13-(4-Methoxyphenyl)-5*H*-dibenzo [b,i] xanthene-5,7,12,14-(13*H*)-tetraone [Table-3, entry 3, Code No. KNP 115].

^{1}H NMR (400 MHz, DMSO-d6):δ 3.69 (s,3H), 6.63 (s, 1H), 6.73–7.99 (m, 12H).^{13}C NMR (400 MHz, DMSO-d6): (ppm) 32.20, 54.82, 113.10, 122.67, 124.99, 125.64, 127.73, 130.89, 131.75, 133.13, 133.27, 133.66,156.71,183.59. FT-IR ν_{max} cm^{-1} : 3090, 1670, 1595, 1176,727.
Red powder (80%), m.p. 312 - 314 ºC

13-(4-Bromophenyl)-5*H*-dibenzo[b,i] xanthene-5,7,12,14-(13*H*)-tetraone [Table-3, entry 4, Code No. KNP 116].

^{1}H NMR (400 MHz, DMSO-d6): δ 6.63 (s, 1H), 6.91–7.94 (m, 12H).FT-IR ν_{max} cm^{-1} : 3090, 1667, 1595, 1277, 1087,729.
Brown powder (88%), m.p. 334 - 336 ^{o}C

13-(4-Nitrophenyl)-5*H*-dibenzo[b,i] xanthene-5,7,12,14-(13*H*)-tetraone [Table-3, entry 5, Code No. KNP 117].

^{1}H NMR (400 MHz, DMSO-d6): δ 6.65 (s, 1H), 7.10–7.99 (m, 12H). FT-IR ν_{max} cm^{-1} : 3092, 1671, 1564, 1485, 1354, 1153, 740.
Orange powder (87%), m.p. 328 - 330 ^{o}C

13-(4-Fluorophenyl)-5*H*-dibenzo [b,i] xanthene-5,7,12,14-(13*H*)-tetraone [Table-3, entry 6, Code No. KNP 118].

^{1}H NMR (400 MHz, DMSO-d6):δ 6.68 (s, 1H), 6.95–7.99 (m, 12H).^{13}C NMR (100 MHz, DMSO-d6): (ppm) 32.33, 114.08, 114.29, 122.29, 125.03, 125.66, 128.42, 128.49, 130.92, 131.80, 133.23, 133.69, 137.30, 158.85, 161.23, 182.28, 183.53.FT-IR ν_{max} cm^{-1} : 3090, 1670, 1564, 1153, 727.
Brown powder (85%), m.p. 276- 278 ºC.

13-phenyl-5*H*-dibenzo[b,i]xanthene-5,7,12,14-(13*H*)-tetraone [Table-3, entry 7, Code No. KNP 119].

^{1}H NMR (400 MHz, DMSO-d6): δ 6.68 (s, 1H), 7.10–8.13 (m, 13H).^{13}C NMR (400 MHz,.FT-IR ν_{max} cm^{-1} : 3091, 1677, 1574, 1173, 745.
Red powder (88%), m.p. 305 - 307 ^{o}C

13-(3-Nitrophenyl)-5*H*-dibenzo[b,i] xanthene-5,7,12,14-(13*H*)-tetraone [Table-3, entry 8, Code No. KNP 120].

^{1}H NMR (400 MHz, DMSO-d6): δ 6.66 (s, 1H), 7.10–7.97 (m, 12H). FT-IR ν_{max} cm^{-1} : 3085, 1672, 1574, 1181, 747.
Orange powder (85%), m.p. 340 - 342 ^{o}C

Antibacterial Susceptibility Assay by MIC Test:

Antibacterial susceptibility assay was carried out by MIC test. Where in two bacterial pathogens E.coli and *Staphylococcus aureus* was used.MIC test is carried out by antimicrobial susceptibility testing protocol, Schwalve, Moore and Goodwin, Crc Press 2007. 13-aryl-5*H* dibenzo [b, i] xanthenes 5, 7, 12, 14 (13*H*)-tetraones shows fairly good antibacterial activity compared to ciprofloxacin as standard compound.

Anti-inflammatory Assay using Gelatin Zymography:

Anti-inflammatory assay carried out by the detection of MMP-2 and MMP-9 using

Gelatine Zymography.13-aryl-5*H* dibenzo [b, i] xanthenes 5, 7, 12, 14 (13*H*)-tetraones shows fairly good anti-inflammatory activity compared to positive control of tetracycline hydrochloride.

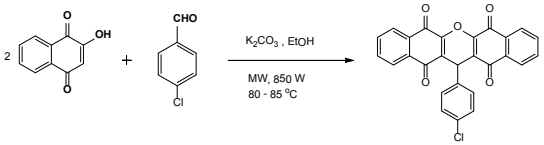
Results and Discussions

Optimization of reaction conditions

Optimization of catalyst loading

To find optimal loading of catalyst and reaction condition, a mixture of 2-hydroxy 1,4-naphthaquinone (2mmol), 4-chlorobenzaldehyde (1 mmol) and K_2CO_3 as solid base catalyst in ethanol (3 ml) as solvent were irradiated in microwave synthesizer system at 850W (80°C-85°C) for 180 sec as model reaction. In the absence of catalyst, the yield of the product was very low which indicate crucial role of catalyst. 20 mol% of K_2CO_3 as catalyst was suitable to catalyze the reaction smoothly further increment of the catalyst did not lead to any significant changes in the reaction yield, results are given in **Table 1.**

Table 1 Optimization study for the amount of K_2CO_3



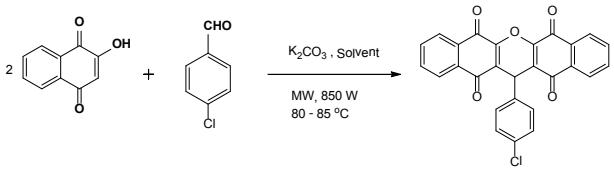
Entry	Catalyst (mol %)	Reaction Time (sec)	Yield [b] (%)
1	0	180	10
2	2	180	35
3	5	180	50
4	10	180	65
5	15	180	80
6	**20**	**180**	**90**
7	25	180	88

Reaction conditions: 2-hydroxy 1,4-naphthaquinone (2mmol), 4-chlorobenzaldehyde (1 mmol) and K_2CO_3 in ethanol(3 mL) were stirred at 850W

[a] Isolated yield of purified products

The effect of different solvents on the yield of model reaction was studied and results are given in **Table 2**. In ethanol, the reaction proceeds very smoothly with high yield. While in MeOH, CH_3CN, DMF, H_2O reaction proceeds with lower yields as compared to EtOH.

Table 2 Optimization of the reaction conditions using different solvents



Entry	Solvent	Reaction Time (sec)	Yield [b] (%)
1	**EtOH**	**180**	**90**
2	MeOH	180	70
3	CH_3CN	180	35
4	DMF	180	45
5	H_2O	180	60

Reaction conditions: 2-hydroxy 1,4-naphthaquinone (2mmol), 4-chlorobenzaldehyde (1 mmol) and K_2CO_3 (20 mol %) in solvent (3 mL) were stirred at 850W

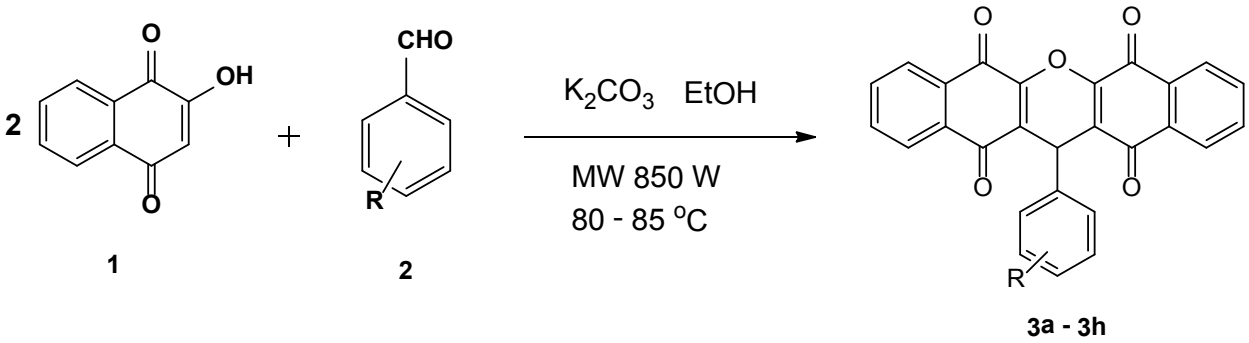
[a] Isolated yield of purified products

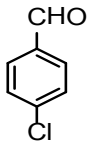
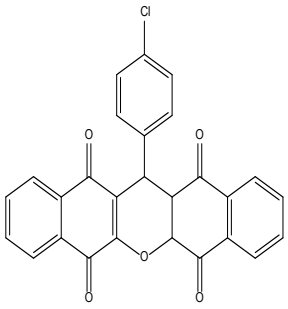
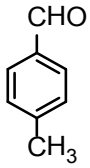
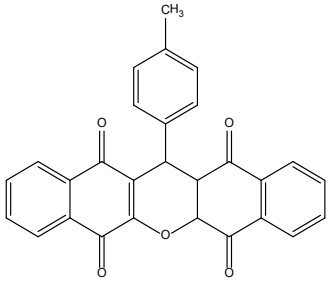
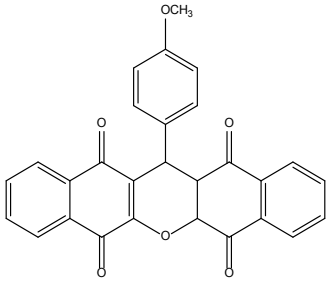
To study the substrate scope, optimized reaction conditions were applied to various aromatic aldehydes with 2-hydroxy 1, 4-naphthaquinone in microwave synthesizer system. Substrates with various functionalities reacted well and afforded high yield of the desired 5H-dibenzo [b, i] xanthene-tetraones derivatives within shorter reaction time and results are given in **Table 3.**

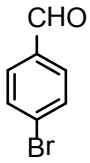
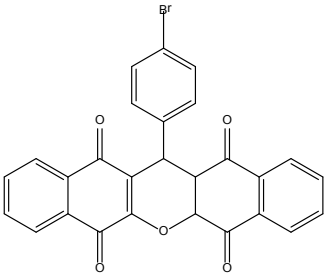
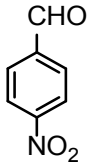
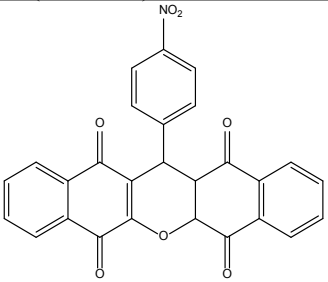
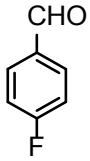
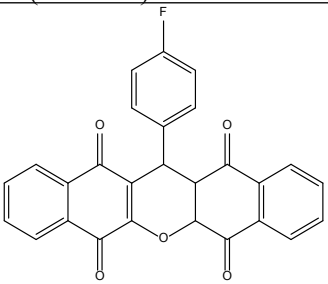
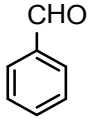
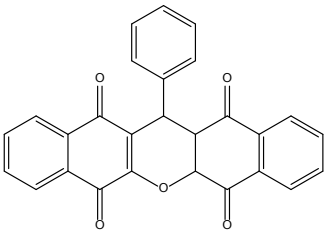
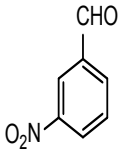
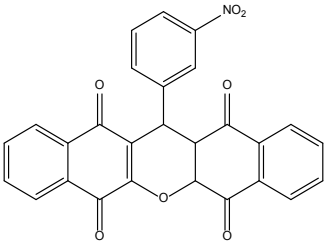
Mechanism of formation of compounds

The plausible mechanism for the K_2CO_3 catalysed synthesis of 5H-dibenzo [b, i]

Table 3 K_2CO_3 catalysed reaction of 2-hydroxy 1, 4-naphthaquinone and various aryl aldehydes



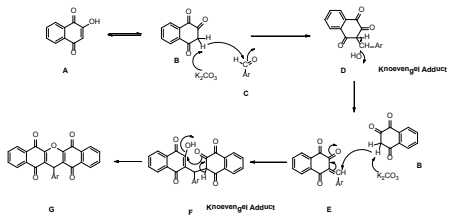
Entries	Aryl Aldehyde	Product	M.P. ^{0}C (References)	Microwave Heating	
				Time /sec	Yield [b] (%)
1		 3a(KNP 103)	330-332 [19,22]	180	90
2		 3b(KNP 105)	304-306 [19,22]	180	82
3		 3c(KNP 115)	312-314 [19,22]	180	80

4		 3d(KNP116)	334-336 [19,22]	180	88
5		 3e(KNP117)	328-330 [19,22]	180	87
6		 3f(KNP 118)	276-278 [19,22]	180	85
7		 3g(KNP 119)	306-308 [19,22]	180	88
8		 3h(KNP120)	340 – 342 [19,22]	180	85

Reaction conditions: 2-hydroxy 1,4-naphthaquinone (2mmol), aromatic aldehyde (1 mmol) and K_2CO_3 (20 mol %) in ethanol (3 mL) were stirred at 850W.

[a] Isolated yield of purified products

xanthene-tetraones derivatives is depicted in **scheme 2**. 2-hydroxy 1,4-naphthaquinone exist in its tautomeric form as B. K_2CO_3 abstract proton from active methylene group of B and further undergoes Knoevenagel condensation with aromatic aldehyde (C) to give Knoevenagel adduct (D).The formed adduct D in the presence of K_2CO_3 undergoes dehydration to give compound (E).Another molecule of naphthoquinone B in the presence of base undergoes Knoevenagel condensation with E to give the another Knoevenagel adduct as F which followed by cylclization and dehydration, affords the desired product G **(Scheme 2).**



Scheme 2 Plausible mechanistic pathway for the synthesis of 5H-dibenzo [b, i] xanthene-tetraones

Antibacterial Activity

The antibacterial assays were carried out by the dilution method using the following bacterial strains: Staphylococcus aureus and Escherichia Coli. Antibacterial Susceptibility Assay by MIC Test of synthesized samples 3a, 3b and 3e were carried out. KNP 103(3a), KNP 116(3d) and KNP 117(3e) shows highly potent antibacterial activity against Gram +Ve bacteria such as *Staphylococcus aureus* which was shown in **Table 4**.

Anti-inflammatory Activity

Anti-inflammatory assay of selected samples KNP 116(3d) and KNP 117(3e) were carried out using Gelatine Zymography.KNP 116(3d) and KNP 117(3e) shows moderately to good anti inflammatory susceptibility in the lower band MMP-2 (Gelatinases –A) as compared to MMP-9 (Gelatinases –B) which was shown in **Table 5**.

Table 4 Anti-bacterial results of 13-aryl-5*H* dibenzo [b, i] xanthenes 5, 7, 12, 14 (13*H*)-tetraones

Sl. No.	Samples	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/ml	3.12 mg/ml	1.6 mg/ml	0.8 mg/ml	0.4 mg/ml	0.2 mg/ml
	E.coli										
01	KNP-103	S	R	R	R	R	R	R	R	R	R
02	KNP-116	S	S	S	R	R	R	R	R	R	R
03	KNP-117	S	S	R	R	R	R	R	R	R	R
	Staph										
01	KNP-103	S	S	S	S	S	R	R	R	R	R
02	KNP-116	S	S	S	S	R	R	R	R	R	R
03	KNP-117	S	S	S	S	S	R	R	R	R	R

Where S = Sensitive, R = Resistant

Table 5 Anti-inflammatory results of 13-aryl-5*H* dibenzo [b, i] xanthenes 5, 7, 12, 14 (13*H*)-tetraones

Sr No	Sample Name	Band (%) MMP-2 (Lower bands)	Band (%) MMP-9 (Upper bands)
1	KNP116	98	88
2	KNP117	68	50
3	PC	100	98
4	NC	10	NIL

Where- PC - POSITIVE CONTROL: - tetracycline hydrochloride

NC - NEGATIVE CONTROL: - MMP sample.

The anti-inflammatory activity has been represented graphically in **Figure 1**

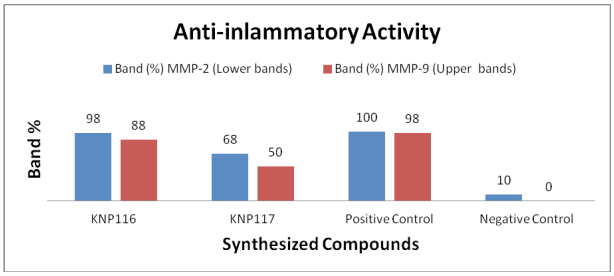


Fig. 1 Anti-inflammatory activity of 13-aryl-5*H* dibenzo [b, i] xanthenes 5, 7, 12, 14 (13*H*)-tetraones

Conclusion

In conclusion, an efficient protocol has been developed for the construction of various 5H-dibenzo [b, i] xanthene-tetraones using basic K_2CO_3 as catalyst via one pot three component addition of 2-hydroxy 1,4-naphthaquinone and aromatic aldehyde under microwave irradiation. 5H-dibenzo [b, i] xanthene-tetraones shows fairly good antibacterial activity compared to *S. Aureus*. The anti-inflammatory activities were evaluated using Gelatine Zymography.13-(4-Bromophenyl)-5*H*-dibenzo[b,i]xanthene-5,7,12,14-(13*H*)-tetraone **(**KNP116) have better efficacy against MMP-2.Clean reaction, low reaction times were the main advantages of this method. Satisfactory yield of products and easy workup make this a useful protocol for green synthesis of this class of compounds.

Acknowledgements

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